

Tobamovirus Expression Vectors

TMV



TMV-Expression Vector

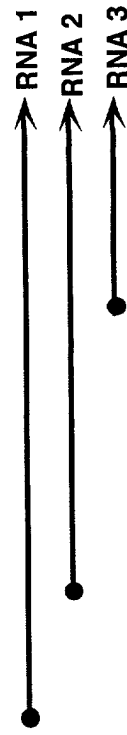


FIG. 1

Tobamovirus Vector for rGal-A Expression

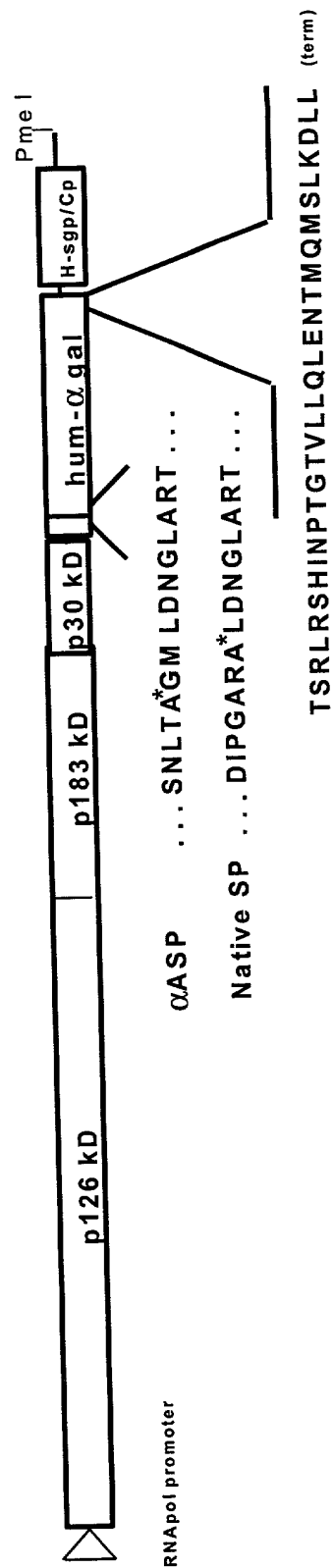


FIG. 2

Accumulation and Activity of WT rGal-A

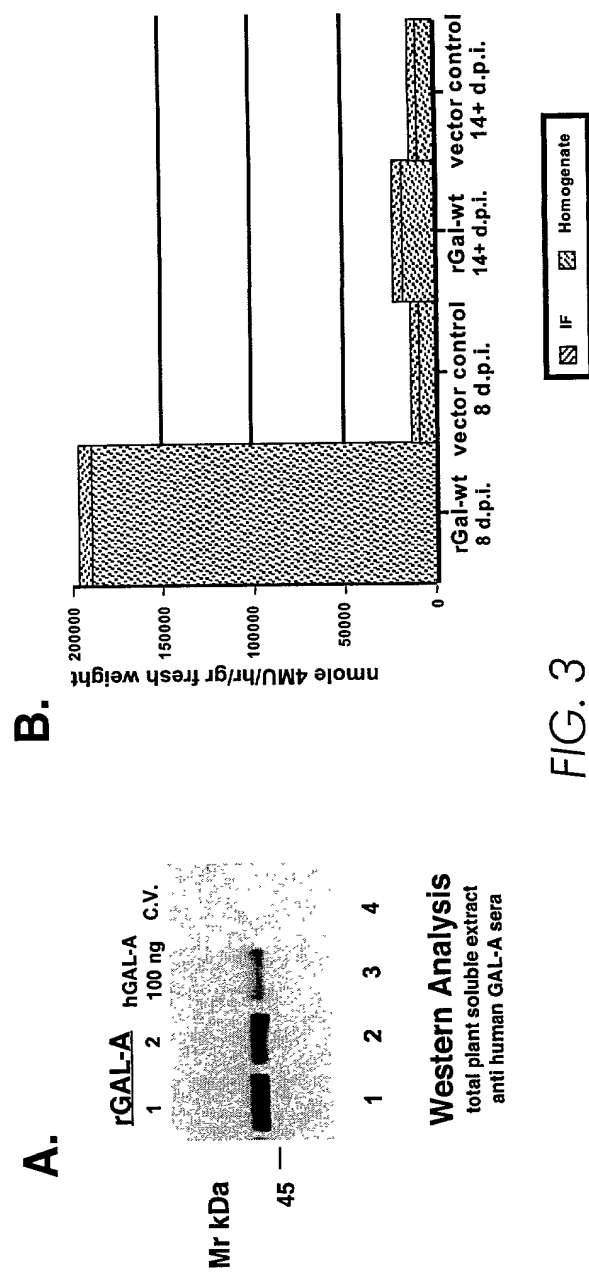


FIG. 3

Accumulation and Activity of WT and ER-Targeted rGal-A

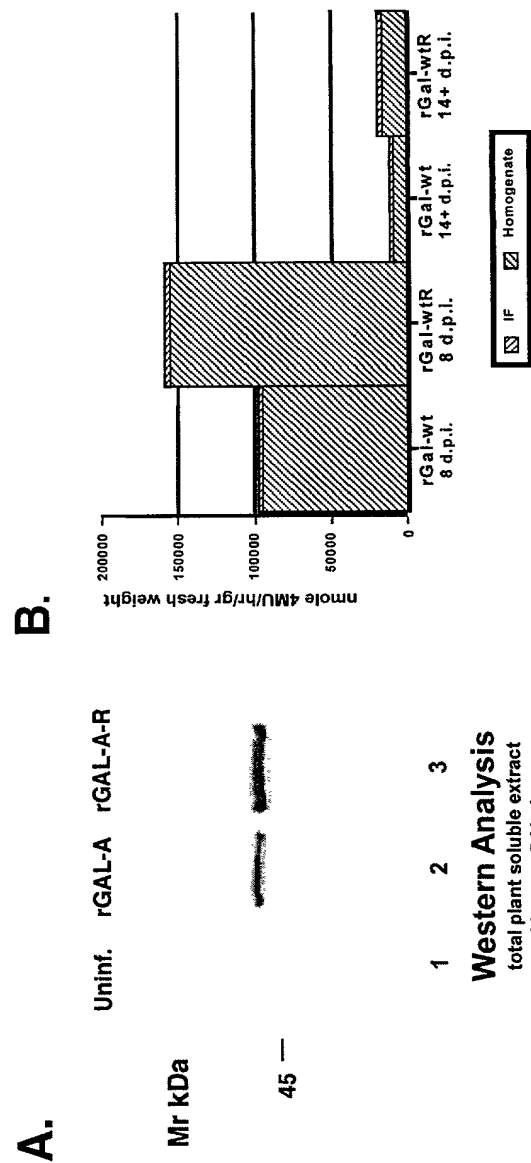


FIG. 4

Carboxy-Modifications to rGal-A

	-30	-20	-10
WT rGAL-A	TSRLRSHINPTGTVLLQL	ENTMQMSLKDLL	
WT rGAL-AR	TSRLRSHINPTGTVLLQL	ENTMQMSLKDLLSEKDEL	
rGAL-4	TSRLRSHINPTGTVLLQL	ENTMQMSL	
rGAL-4R	TSRLRSHINPTGTVLLQL	ENTMQMSLKDEL	
rGAL-8	TSRLRSHINPTGTVLLQL	ENTM	
rGAL-8R	TSRLRSHINPTGTVLLQL	ENTMSEKDEL	
rGAL-12	TSRLRSHINPTGTVLLQL		
rGAL-12R	TSRLRSHINPTGTVLLQL	SEKDEL	
rGAL-25	TSRLR		
rGAL-25R	TSRLRSEKDEL		
Control virus (GFP, AMP, IFN γ)			

* potential CTPP cleavage (Gene 58:177,1987).

FIG. 5

Western Blot Analysis of Carboxy-modified rGal-A

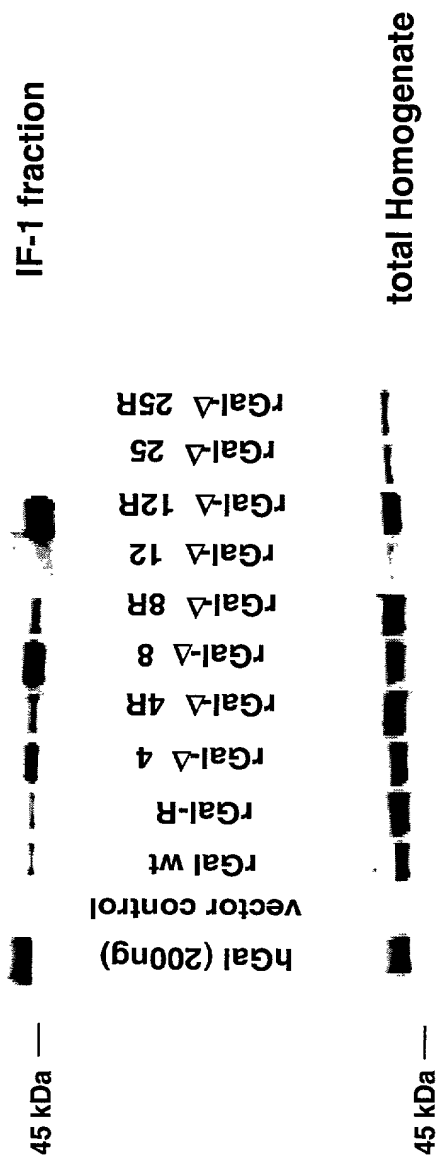


FIG. 6

Enzymatic Activity of Carboxy-Modified rGal-A

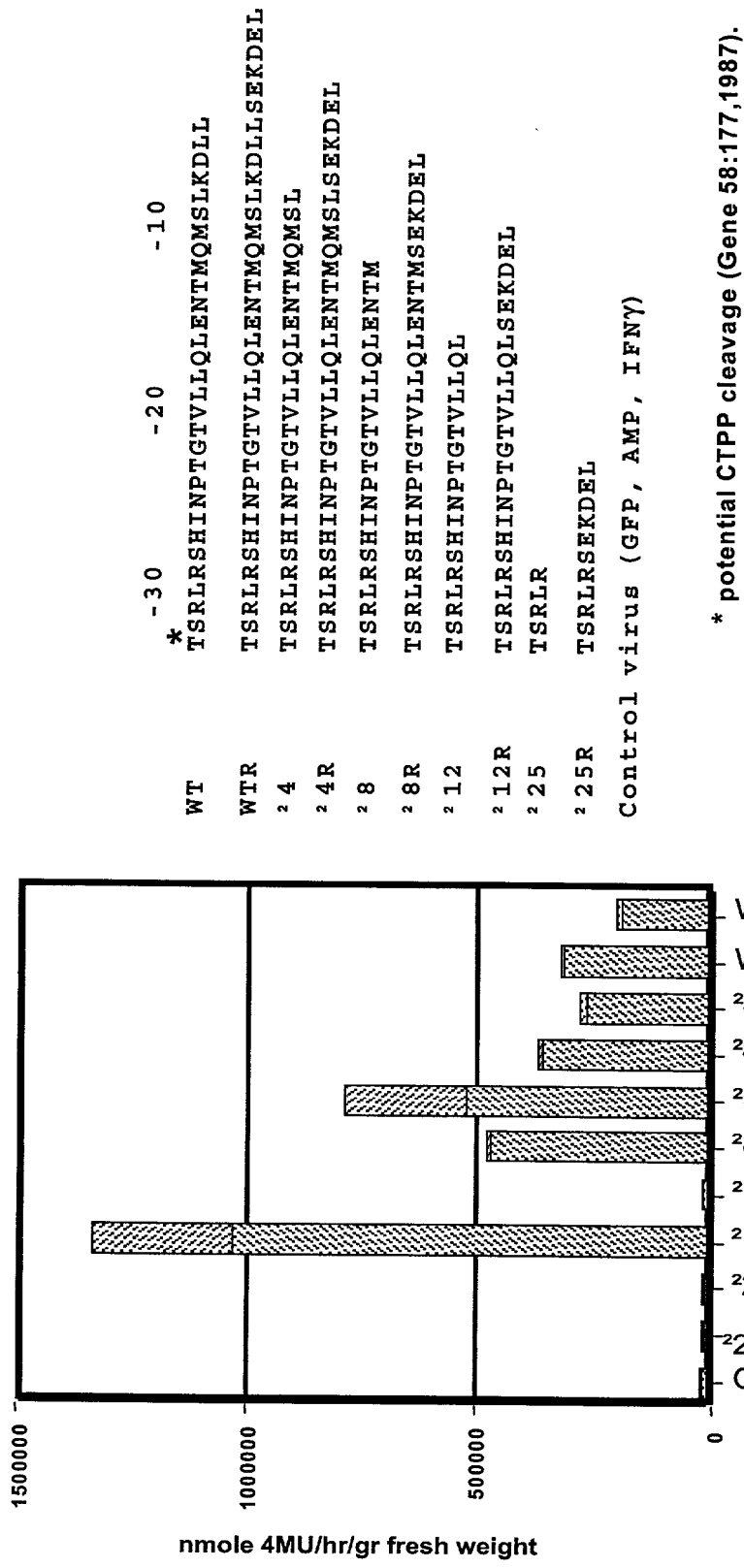


FIG. 7

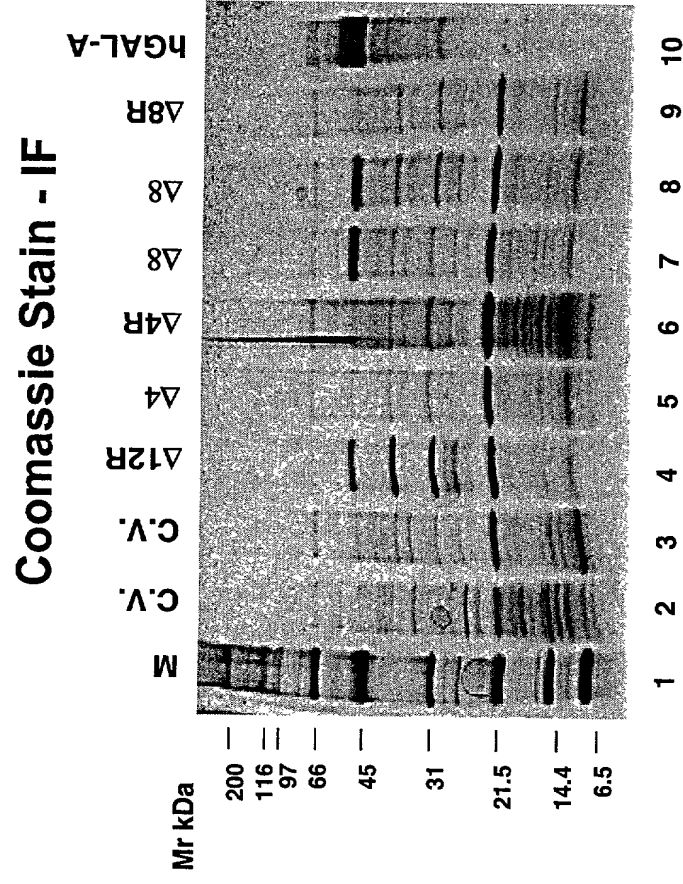


FIG. 8

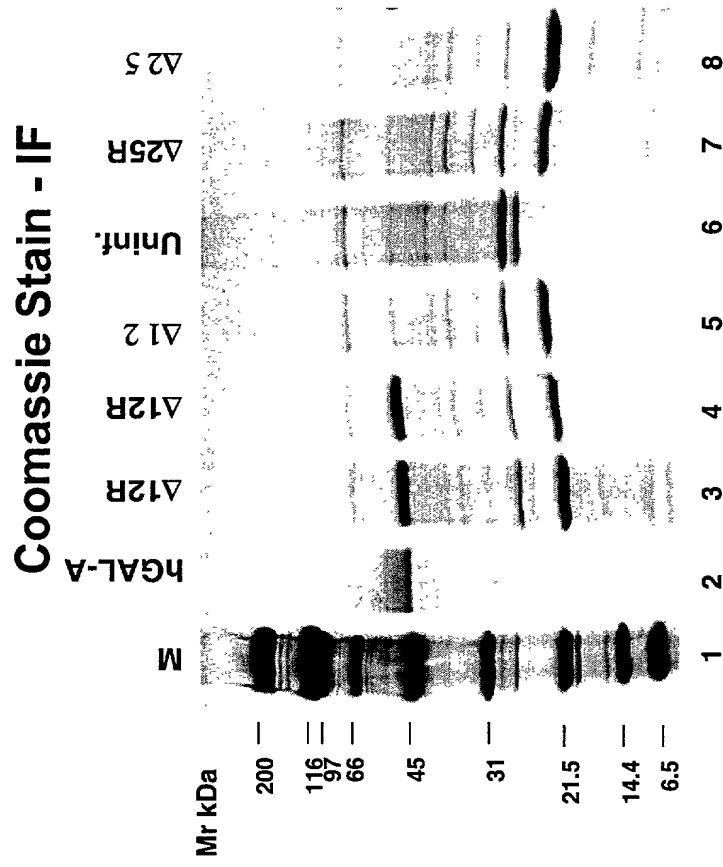


FIG. 9

Schematic of rGal-A Secretion

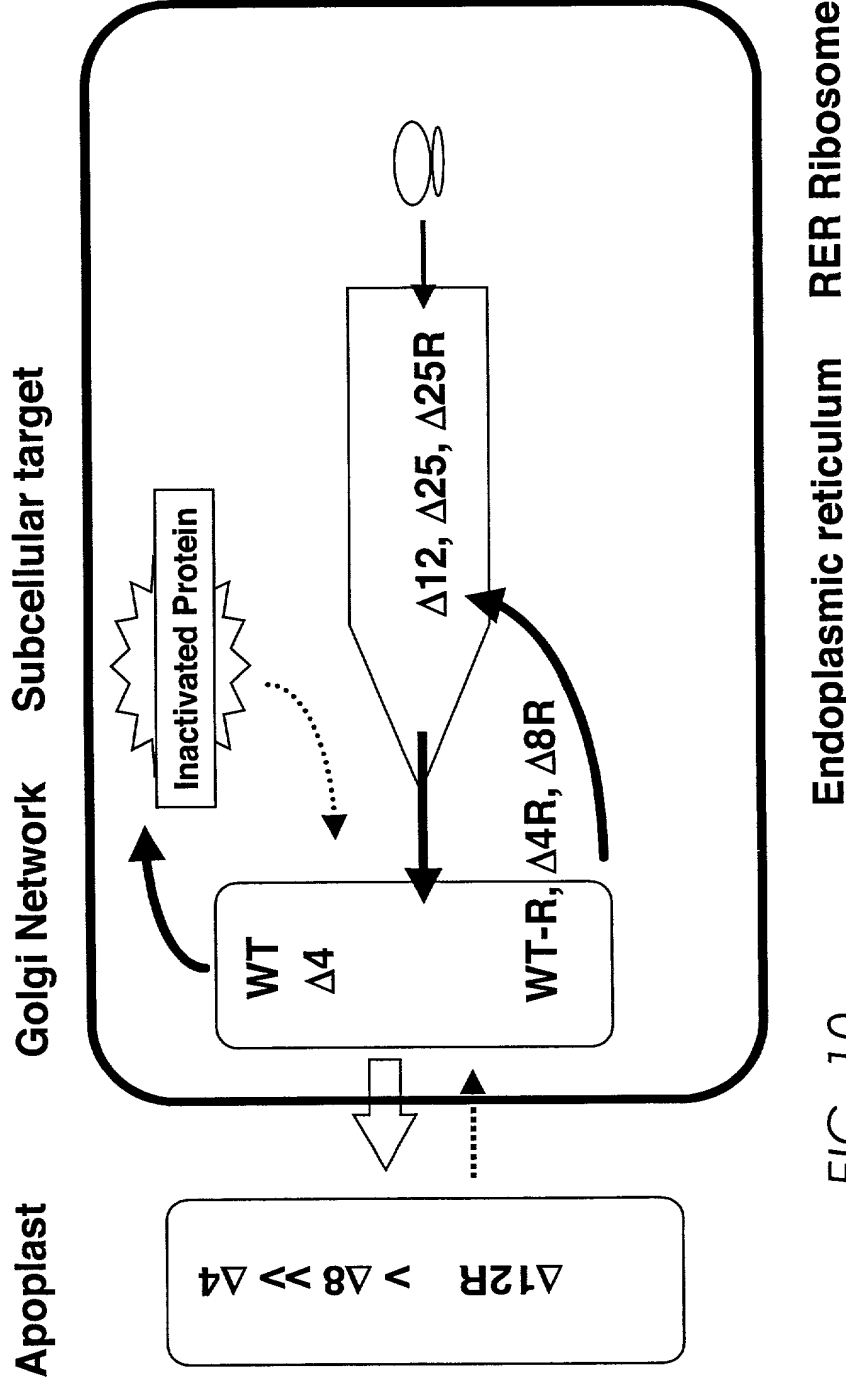


FIG. 10

FIG. 11

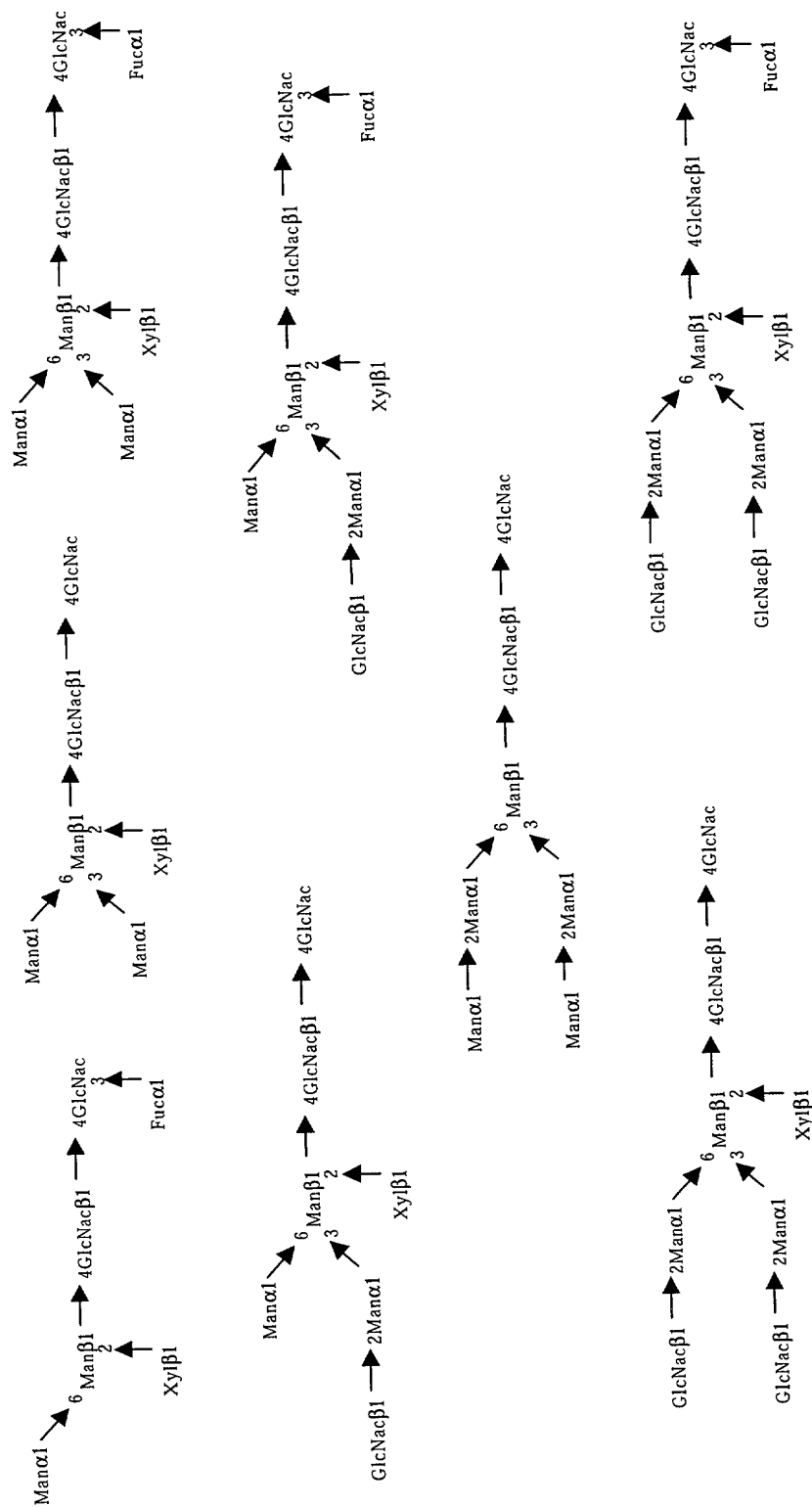


FIG. 12-1

GTATTTTACAAATTACCAACAACAACAACAACAACATTACAATTACTATTTACAATTACAATGGCATACACA
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AGACGCTTATTGCTACCCGGGCGTATCCAGAATTCCAAATTACATTTTATAACACGCAAAATGCCGTGCATTTCGCTTGCA
GGTGGATTGCGATCTTTAGAACTGGAATATCTGATGATGCAAAATCCCTACGGATCATTGACTTATGACATAGGCGGGAA
TTTTGCATCGCATCTGTTCAAGGGACGAGCATATGTACACTGCTGTATGCCCAACCTGGACGTTCCGAGACATCATCGCGC
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GAAGCATTTGACAGATACGCAGAAAATTCCTGAAGACGCTGTCTGTACAATACTTTCCAGACAATGCGACATCAGCCGAT
GCAGCAATCAGGCAGAGTGTATGCCATTGCGCTACACAGCATATATGACATAACCAGCCGATGAGTTCGGGGCGGCACTCT
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CGTTGTTTGTAGTACTAGGCAATTACTGGACAGTGTGATTTCGAGCAGATTTTGTGTTTTTCAAGAAAAGACACCA
CGCGAGATTGAGGATTTCTTCGGAGATCTCGACAGTCTATGTCCGATGGATGCTTGGAGCTGGATATATCAAAATACGA

FIG. 12-1

FIG. 12-2

CAAATCTCAGAATGAATTCCACTGTGCAGTAGAATACGAGATCTGGCGAAGATTGGGTTTTGAAGACTTCTTGGGAGAAG
 TTTGGAAACAAGGGCATAGAAAGACCACCCTCAAGGATTATACCGCAGGTATAAAACTTGCATCTGGTATCAAGAAAAG
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 GAGTGGGTTATACGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTGCCCCGAAGAACGTTTTCCAATGATG
 AGCATTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTGTGACGCGGGCAAGAGCAACTCGGTGCGCGCATACA
 CTATTCTCAGAATGACTTGGTTGAGTACTACCACTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTAT

FIG. 12-2

FIG. 12-3

GCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACC
GCTTTTTTGCACAACATGGGGGATCATGTAACCTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGA
CGAGCGTGACACCACGATGCCTGCAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTT
CCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGG
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AGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTCTGCGCGTAATCTGCTGCTTGCAAAACAAAAAAC
CACCCTACACGCGGTGGTTTGGTTGCGCGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACGGCTTCAGCAGAGCG
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CGCTCTGCTAATCCTGTTACCACTGGCTGCTGCCAGTGGCGATAAGTCTGTCTTACCGGTTGGACTCAAGACGATAGT
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GGGTAACAACCTGGCGGTATGGATGCGGCGGACCAGAGAAAAATCACTCAGGGTCAATGCCAGCGCTTCGTTAATACAG
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FIG. 13-1

GTATTTTACAACAATTACCAACAACAACAACAACAGACAACATTACAATTACTATTTACAATTACAATGGCATAACA
CAGACAGCTACCACATCAGCTTTGCTGGACACTGTCCGAGGAAACAACCTCTGGTCAATGATCTAGCAAAGCGTCGTCT
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GGCAGGCGAGCATTAGAGATCAGGGTGCCTGATCTATATGTGACCTTCCACGACAGATTAGTGACTGAGTACAAGGCCT
CTGTGGACATGCCTGCGCTTGACATTAGGAAGAAGATGGAAGAAACGGAAGTGATGTACAATGCACTTTGAGAATTATCG
GTGTTAAGGGAGTCTGACAAATTCGATGTTGATGTTTTTCCAGATGTGCCAATCTTTGGAAGTTGACCCAATGACGGC
AGCGAAGGTTATAGTCGCGGTGATGAGCAATGAGAGCGGTCTGACTCTCACATTTGAACGACCTACTGAGGCGAATGTTG
CGGTAGCTTTACAGGATCAAGAGAAGGCTTCAGAAAGTGCATTGGTAGTTACCTCAAGAGAAGTTGAAGAACCGTCCATG
AAGGGTTCGATGCCAGAGGAGAGTTACAATTAGCTGCTCTTGCTGGAGATCATCCGAATCGTCTATTCTAAGAACGA
GGAGATAGAGTCTTTAGAGCAGTTTCATATGGCGACGGCAGATTTCGTTAATTCGTAAGCAGATGAGCTCGATTGTGTACA
CGGTCGGATTAAGATTTCAGCAATGAAAACTTTATCGATAGCCTGGTAGCATCACTATCTGCTGCGGTGTCGAATCTC
GTCAAGATCCTCAAAGATACAGCTGCTATTGACCTTGAACCCGCTCAAAAGTTGGAGTCTTGGATGTTGCATCTAGGAA
GTGTTAATCAAACCAACGGCCAAGAGTCTATGCATGGGGTGTGTTGAAACCCACGCGAGGAGTATCATGTGGCGCTTT
TGGAATATGATGAGCAGGGTGTGTTGACATGCGATGATTGGAGAAGAGTAGCTGTTAGCTCTGAGTCTGTTGTTTATTCC
GACATGGCGAACTCAGAACTCTGCGCAGACTGCTTGAACCGGAGAACCGCATGTCAGTAGCGCAAAGGTTGTTCTTGT
GGAGGAGTTCCGGGCTGTGGAACCAAGAAATTTCTTCCAGGGTAAATTTGATGAAGATCTAATTTTAGTACCTG
GGAAGCAAGCCGCGAAATGATCAGAAAGCTGCGAATTCCTCAGGGATTATGTGCCCAGGAAGGACAACGTTAAACCC
GTTGATCTTTTCATGATGAATTTTGGGAAAGCACACGCTGTGAGTTCAAGAGGTTATTCATTGATGAAGGTTGATGTT
GCATACTGGTTGTGTTAATTTTCTTGTGCGGATGTCATTGTGCGAAATTCATATGTTTACGGAGACACACAGCAGATT
CATACATCAATAGAGTTTCAGGATTCCCGTACCCCGCCATTTTGCCAAATGGAAGTTGACGAGGTGGAGACACGCAGA
ACTACTCTCCGTTGTCAGCGGATGTCACACATTATCTGAACAGGAGATATGAGGGCTTTGTCATGAGCACTTCTTCGGT
TAAAAAGTCTGTTTCCAGGAGATGGTCCGGGAGCGCGGTGATCAATCCGATCTCAAAACCTTGCATGGCAAGATCC
TGACTTTTACCAATCGGATAAAGAAGCTGTGCTTTCAAGAGGTTATTCAGATGTTCACTCTGCATGAAGTGAAGGC
GAGACATACTCTGATGTTTCACTAGTTAGGTTAACCCCTACACCGGTCTCCATCATTGCAGGAGACAGCCACATGTTTT
GGTGCATTGTCAAGGCACACCTGTTGCTCAAGTACTACACTGTTGTTATGGATCCTTTAGTTAGTATCATTAGAGATC
TAGAGAACTTAGCTCGTACTGTTAGATATGTATAAGGTCGATGCAGGAACACAATAGCAATTACAGATTGACTCGGTG
TTCAAAGGTTCCAATCTTTTGTGCGAGCGCCAAAGACTGGTGATATTTCTGATATGCAGTTTACTATGATAAGTGTCT
CCCAGGCAACAGCACCATGATGAATAATTTTGTGCTGTTACCATGAGGTTGACTGACATTTTATTGAATGTCAAAGATT
GCATATTGGATATGCTAAGTCTGTTGCTGCACCTAAGGATCAAATCAAACCACTAATACCTATGGTACGAACGGCGGCA
GAAATGCCACGCAGACTGGACTATTGGAAAATTTAGTGCGGATGATTAAAGAAAACCTTAAACGCAACCCGAGTTGCTGG
CATCATTGATATTGAAAATACTGCATCTTTGGTTGTAGATAAGTTTTTTGATAGTTATTTGCTTAAAGAAAAAGAAAAC
CAATAAAAAATGTTTCTTTGTTGATGAGAGTCTCTCAATAGATGCTTAGAAAAGCAGGAACAGGTAACAATAGGCCAG
CTCGCAGATTTTGTATTTGTGGATTGCGCAGCAGTTGATCAGTACAGACACATGATTAAAGCACAACCCAAACAAAAGTT
GGACACTTCAATCCAAACGGAGTACCGGCTTTGAGACGATTGTGTACCATTTCAAAAAGATCAATGCAATATTGCGCC
CGTTGTTAGTGAGCTTACTAGGCAATTACTGGACAGTGTGATTTCGAGCAGATTTTGTGTTTTCAAGAAGACACCA
CGCAGATTGAGCATTTCTTCGGAGATCTCGACAGTCACTGCGGATGGATGTCTTGGAGCTGCATATACAAAATACGA

FIG. 13-1

FIG. 13-2

CAAAATCTCAGAATGAATTCACCTGTGCAGTAGAATACGAGATCTGGCGAAGATTGGGTTTCGAAGACTTCTTGGGAGAAG
TTTGGAAAACAAGGGCATAGAAAGACCACCTCAAGGATTATACCGCAGGTATAAAAACTTGCATCTGGTATCAAGAAAAG
AGCGGGGACGTACGACGTTTCATTGAAACACTGTGATCATTGCTGCATGTTTGGCCTCGATGCTTCCGATGGAGAAAAT
AATCAAAGGAGCCTTTTTCGGGTGACGATAGTCTGCTGTACTTTCCAAAGGGTTGTGAGTTTCCGGATGTGCAACACTCCG
CGAATCTTATGTGAATTTTGAAGCAAACTGTTTAAAAACAGTATGGATACTTTTGGCGAAGATATGAATACATCAC
GACAGAGGATGCATTGTGATTACGATCCCCTAAAGTTGATCTCGAAACTTGGTGCTAAACACATCAAGGATTGGGAACA
CTTGGAGGAGTTTCAAGAGTCTCTTTGTGATGTTGCTGTTTCCGTTGAACAATGTGCGTATTACACACAGTTGGACGACG
CTGTATGGGAGGTTTATAAGACCGCCCTCCAGGTTCCGTTTGTATATAAAGTCTGGTGAAGTATTGTCTGATAAAGTT
CTTTTTAGAAGTTTGTTTATAGATGGCTCTAGTTGTTAAAGGAAAAGTGAATATCAATGAGTTTATCGACCTGACAAAA
TGGAGAAGATCTTACCGTCGATGTTTACCCCTGTAAGAGTGTATGTGTTCCAAAGTTGATAAAAAATGGTTCATGAG
AATGAGTCATTGTGAGGGGTGAACCTTCTTAAAGCAGTTAAGCTTATTGATAGTGGATACGTTCTGTTAGCCGGTTTGGT
CGTCACGGGCGAGTGGAACTTGCCTGACAATTGCAGAGGAGGTGTGAGCGTGTGTCTGGTGGACAAAAGGATGGAAAAG
CCGACGAGGCCATTCTCGGATCTTACTACACAGCAGCTGCAAGAAAAGATTTCAGTTCAAGGTCGTTCCCAATTATGCT
ATAACCCACCGAGACCGATGAGAAACGCTGCGCAAGTTTATGTTAATATTAGAAATGTGAAGATGTGACGGGTTTCTG
TCCGCTTTCTCTGGAGTTTGTGTCGGTGTGATTGTTTATAGAAATAATATAAAATTAGGTTTGAAGAGAGAAGATTACAA
ACGTGAGAGACGGAGGCCCCATGGAACCTACAGAAAGTCTGTTGATGAGTTCATGAAGATGTCCCTATGTGATCAGG
CTTGCAAAGTTTCGATCTCGAACCGGAAAAAGAGTGATGTCCGCAAGGGGAAAAATAGTAGTAGTGATCGGTGAGTGCC
GAACAAGAACTATAGAAATGTTAAGGATTTTGGAGGAATGAGTTTAAAAAGAATAATTTAATCGATGATGATTCGGAGG
CTACTGTCCCGAATCGGATTCGTTTAAATAGATCTTACAGTATCACTACTCCATCTCAGTTTCGTTCTTGTCTAataa
ttaaaatgcagctgaggaaccagaactacatctgggctgcgcgcttgccgttcgcttcctggccctcgttccctgggac
atccctggggctagagcactggacaatggattggcaaggacgcctaccatgggctggctgcactgggagcgttcatgtg
caaccttgactgccaggaagagccagattcctgcatcagtgagaagctcttcatggagatggcagagctcatggctcag
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cttcaggcagaccctcagcgcttctcatgggattcgccagctagctaattatgttcacagcaaggactgaagctagg
gatttatgcagatgttggaataaaacctgcgcaggcttccctgggagtttggatactacgacattgatgccagacct
ttgctgactggggagtagatctgctaaaatttgatggttggtactgtgacagtttggaatttggcagatggttataag
cacatgtccttggccctgaataggactggcagaagcattgtgtactcctgtgagtgccctctttatgtggccctttca
aaagcccaattatacagaaatccgacagctactgcaatcactggcgaatttggctgacattgatgattcctggaaaagta
taaagagtatcttggactggacatctttaaaccaggagagaattgttgatgttggctggaccagggggttggaaatgacca
gatattgttagtgattggcaacttggcctcagctggaatcagcaagtaactcagatggccctctgggctatcattggctgc
tcctttattcatgtctaatgacctccgacacatcagccctcaagccaaagctctccttcaggataaaggacgtaattgcc
tcaatcaggaccccttgggcaagcaagggtaccagcttagacaggagacaacttgaagtgtgggaacgacctctca
ggcttagcctgggctgtagctatgataaacggcaggagattggtggacctcgtcttataccatcgagttgcttcct
gggtaaaaggagtgccctgtaatcctgcctgcttcatcacacagctcctccctgtgaaaaggagctagggttctatgaat
ggacttcaaggttaagaagtcacataaatcccacaggcactgttttgcttcagctatctgaaaaggacgaattatgacct
aggGGGTAGTCAAGATGCATAATAAATAACGGATTGTGTCCGTAATCACACGTGGTGGTACGATAACGCATAGTGT
TCCCTCCACTTAAATCGAAGGTTGTGCTTGGATCGCGCGGTCAAATGTATAGGTTTCATATACATCCGAGGCACGT
AATAAAGCGAGGGGTTTCGGGTCGAGTCCGCTGTGAAACTCGAAAAGTTCCGGAAAAACAAAAAGAGAGTGGTAGGTAA
TAGTGTTAATAATAAGAAAATAAATAAGTGGTAAGAAAAGTTTGAAGTTGAGGAAATTGAGGATAATGTAAGTGTG
ACGAGTCTATCGCGTCATCGAGTACGTTTAAATCAATATGCCTTATACAATCAACTCTCCGAGCCAATTGTTTACTTAA
GTTCCGCTTATGCAGATCCTGTGCAGCTGATCAATCTGTGTACAAATGCATTGGGTAACCACTTTCAAACGCAACAAGCT
AGGACAACAGTCCAACAGCAATTTGCGGATGCCGGAACCTGTGCCTAGTATGACAGTGAATTTCTGTCATCGGATTT
CTATGTGTATAGATATAATTCGACGCTTGATCCGTTGATCACGGCGTTATTAATAGCTTCGATACTAGAAATAGAATAA
TAGAGGTTGATAATCAACCCGACCGAATACTACTGAAATCGTTAACGCGACTCAGAGGTTAGACGATGCGACTGTAGCT
ATAAGGGCTTCAATCAATAATTTGGCTAATGAACtGGTTCGTGGAAGTGGCaTGTTCATCAAGCAAGCTTTGAGACTG
TAGTGCAGTTGCTGTGACCAACAACCTCCGGCTACTAGctattgttgtagatttctaaaaataaagtcactgaagactta
aaattcagggtggctgataccaaaatcagcagtggtgttgcgtccacttaaatataacgattgtcatatctggatccaac
agttaaacattgtggtgtatactgtggtatggcgtaaaacaacggaaaagtcgctgaagacttaaaattcagggtgg
ctgataccaaaatcagcagtggtgttgcgtccacttaaaataacgattgtcatatctggatccaacagttaaaccatgt
gatggtgtatactgtggtatggcgtaaaacaacggagaggttcgaatcctccctaaccgcggtagcgccca

FIG. 13-2

TRANSGENIC VECTOR FOR rGCB EXPRESSION

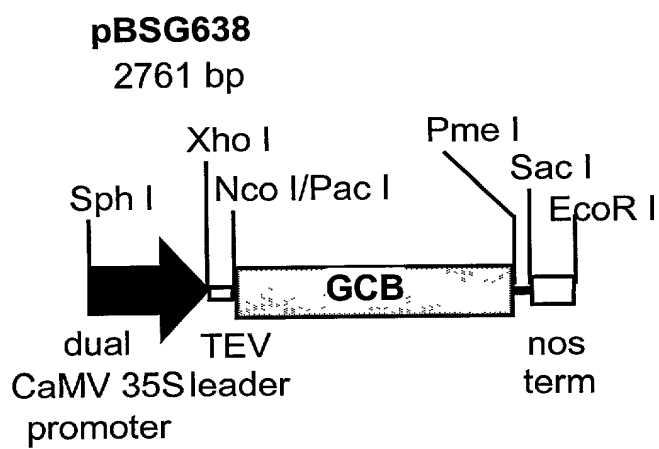


FIG. 14

VIRAL VECTOR FOR rGCB EXPRESSION

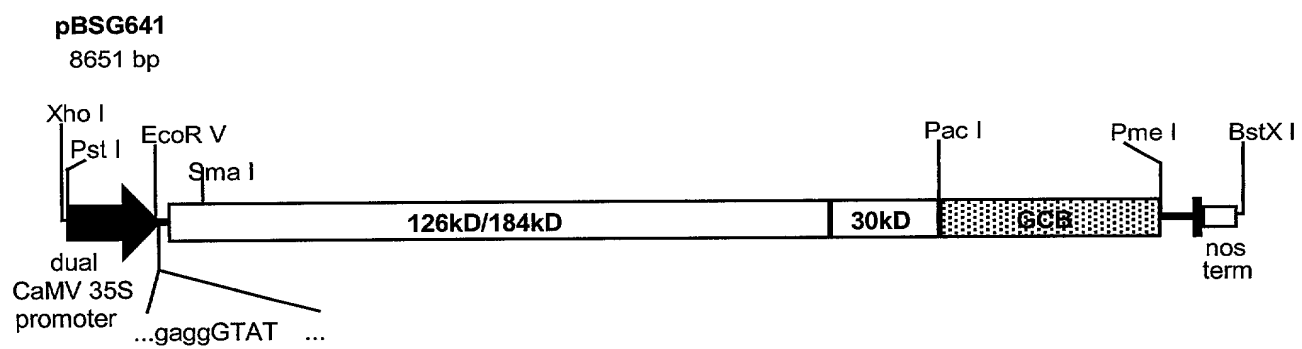


FIG. 15